Subterranean Biology 26: 39–53 (2018)
doi: 10.3897/subtbiol.26.26643
http://subtbiol.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Subterranean Biology
Published by The International Society for Subterranean Biology

# A new cave locality for *Astyanax* cavefish in Sierra de El Abra, Mexico

Luis Espinasa[1], Laurent Legendre[2], Julien Fumey[3], Maryline Blin[4], Sylvie Rétaux[4], Monika Espinasa[5]

**1** *School of Science, Marist College, 3399 North Rd, Poughkeepsie, New York 12601, USA* **2** *UMS AMAGEN, CNRS, INRA, Université Paris-Saclay, Gif-sur-Yvette, France* **3** *Évolution, Génomes, Comportement, Écologie. CNRS, IRD, Univ Paris-Sud. Université Paris-Saclay, 91198 Gif-sur-Yvette, France* **4** *Equipe Développement Evolution du Cerveau Antérieur, Paris-Saclay Institute of Neuroscience, CNRS and University Paris-Sud and Paris-Saclay, 91198 Gif-sur-Yvette, France* **5** *Department of Mathematics, Science, Biology, Engineering and Technology, SUNY Ulster, Stone Ridge, USA*

Corresponding author: *Luis Espinasa* (Luis.espinasa@marist.edu)

Academic editor: *O. Moldovan* | Received 13 May 2018 | Accepted 14 June 2018 | Published 4 July 2018

*http://zoobank.org/29CA8372-4D22-4625-8759-990B8C273E79*

**Citation:** Espinasa L, Legendre L, Fumey J, Blin M, Rétaux S, Espinasa M (2018) A new cave locality for *Astyanax* cavefish in Sierra de El Abra, Mexico. Subterranean Biology 26: 39–53. https://doi.org/10.3897/subtbiol.26.26643

## Abstract

The characiform fish *Astyanax mexicanus* comes in two forms, a surface-dwelling morph which lives in the rivers of North and Central America and a blind, depigmented cave-dwelling morph which inhabits caves in Mexico. In recent years, this species has arguably become among the most influential model system for the study of evolutionary development and genomics in cave biology. While recent articles have analysed in great detail *Astyanax* genetics, development, physiology, phylogeny and behaviour, there have been comparatively few recent studies concerning its ecology and in particular its biogeography. Mitchell et al. (1977) reported the species inhabiting 29 caves in the Sierra de El Abra region. Despite the elapsing of over 40 years and the latest surge of interest in the model, not a single new cave locality had been described for the species. We describe here a new and 30$^{th}$ cave locality, Chiquitita Cave, inhabited by troglomorphic *A. mexicanus*. Their morphology, eye histology, 16S rRNA DNA sequence, and smelling capabilities are analysed. This cave represents the southernmost extension for the cave morph's habitat within the Sierra de El Abra. Its name, Chiquitita Cave (Tiny Cave), was chosen in reference to a potential hydrologic connection to "Chica Cave" (small cave), which is among the most studied populations of *Astyanax*.

## Keywords

*Astyanax*, Chica Cave, Sierra de El Abra, troglomorphy, Troglobite

Copyright *Luis Espinasa et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## Introduction

The blind Mexican tetra *Astyanax mexicanus* De Filippi 1853 has become the main contributor in the understanding of the genetic and developmental controls of troglomorphic features. It is also ranked among the influential model systems in evolutionary developmental (EvoDevo) biology (Jeffery 2001; Jeffery 2012). The cavefish have a conspecific surface-dwelling morph which lives in nearby surface streams throughout most of México. Both the eyed, pigmented surface morph and the eyeless, depigmented cave morph remain inter-fertile, making the species well-suited for experimental manipulations (Jeffery 2012; Elipot et al. 2014).

To date, 29 cave localities have been described for *A. mexicanus* (Mitchell et al. 1977), all of which occur within the El Abra region, in Northern Mexico. Two more cave localities, Granadas and La Joya, have been described for the closely related species of *Astyanax aeneus* in Guerrero, Southern Mexico (Espinasa et al. 2001; Kopp et al. 2017).

The cave morph was first discovered in Chica Cave by Salvador Coronado in 1936 (Hubbs and Innes 1936). The New York Aquarium expedition of Breder and associates in 1940 produced a map of the cave, a description of the environment, and the peculiarities of the population that inhabits this cave. A small entrance (1.5 by 3.5 m) is found at the base of a stream bed that is dry throughout the year except during particularly large storms in the rainy season. This stream bed has no surface fish. The cave is 591 m long and in a step-wise fashion the passage continuously pitches downward through a series of couple meter deep pits. Four main pools are encountered (Pool I-IV), with the lowest pool IV, the final sump, lying at 19 m below the entrance level (Mitchell et al. 1977; Elliott 2015).

The Chica Cave population is a highly varied one, being comprised of everything from typical eyeless, depigmented cave fish to typical surface fish. Since depigmented fish with eyes and pigmented fish without eyes can be found, introgression has been suggested (Avise and Selander 1972). Interestingly, pool I, which is the one closest to the entrance, has the highest proportion of troglomorphic fish, while pool IV, which is the deepest and furthest from the entrance has the largest number of epigeomorphic fish. Mitchell et al. (1977) have argued that surface fish enter the cave not through its higher entrance, which is a dry stream bed during large parts of the year, but that instead surface fish swim in from a spring adjoining Tampaón river, reaching the lower and deeper pool IV of Chica cave. The final sump and Tampaón river are only 972 m apart. Pool IV's final sump is at an elevation of 31 m above sea level. Tampaón river is at 28.5 m. Thus the terminal pool is only 2.5 meters higher than the surface river. Further support for a surface connection is that these authors have found cichlid fish in the deeper pools, which are clearly surface river fish that must have swam in.

The Chica Cave population was included in the first genetic study conducted on cave *Astyanax* (Avise and Selander 1972). They used isoenzymes. From this study a hypothesis to explain the mixed nature of the population was born. They observed that

the Esterase 2d haplotype was fixed (Pachón, Sabinos) or in high frequencies (Chica) in cave populations and absent from surface populations and the haplotype Glutamate Oxalate Transaminase 2d was fixed (Sabinos) or in high frequencies (Chica) in cave population and essentially absent from surface populations (the exception were only 2 individuals out of 257 assessed ie. 0.78%). When they divided the Chica individuals into eyeless, sunken eye and eyed individuals, from most troglomorphic to most epigeomorphic, they found a clear correlation between frequencies of homozygous individuals for the cave allele, heterozygous and homozygous for the surface allele, according to morphology. They concluded that "individuals from surface populations have recently entered the cave and are interbreeding with eyeless individuals" (Avise and Selander 1972), confirming with genetic evidence that surface fish probably regularly enter the Chica cave.

Mitchell et al. (1977) discussed the most likely point of entrance of fish from Tampaón river into the Cueva Chica system. They argued for a trio of small, closely associated, "tinajas" (water-filled depressions) located in the eastern part of El Pujal town and about 225 m north of Tampaón river. From these tinajas a rather prominent arroyo enters the nearby channel of Tampaón river. At high flood stage, the Tampaón river fills this stream to the tinajas, which like the final sump of Chica are only about 3 m above the Tampaón river.

The purpose of this paper is to describe a new cave population of troglomorphic fish discovered near these tinajas as well as the cave environment they inhabit. Of relevance is that since 1977, this has been the first new *Astyanax* cave locality discovered in the Sierra de El Abra.

## Methods

The initial purpose of this study was to observe the *Astyanax* fish population at the tinajas described by Mitchell et al. (1977) as the potential resurgence of Chica caves in hopes of finding hybrid fish. While at the town of El Pujal, local inhabitants pointed out the presence of a well just 45 m away from the tinajas. From this well, water is extracted for use by the town dwellers. Upon inspection, it appeared that the well was not an artificially excavated well but instead was a natural 5.7 m deep pit that reached a large pool of water. A pipe had been installed and water is extracted via a pump located inside the adjoining work house. Twenty-five meters from this well and in the direction of the tinajas, a small hole was found among the roots of a large tree. This hole lead to a 4 m long chamber, which reached water level. From it a poecilid, an epigeomorphic *Astyanax* and two troglomorphic *Astyanax* fish were collected. The following day, the pit of the well was descended with the help of a cable ladder. The pool was examined with the help of a scuba diving lamp, mask and snorkel. A school of about 20 epigeomorphic *Astyanax* was seen as well as some troglomorphic *Astyanax*. Four more specimens were collected. All specimens were in the twilight area.

## Cave

Topographic map of main chamber of the well and the small cave under the tree was made with tape and a Suunto compass and clinometer on 3/23/16. Croquis map of a side gallery of the well was made on 5/26/16 when water level was significantly lower and allowed for exploration. Although the less than 20 m of passage between the well and the small cave remain unconnected by human exploration, it is suggested they form part of a single system and thus the well and the small cave are referred here both as "Chiquitita Cave" (Fig. 1). Chiquitita cave is within the town of El Pujal, San Luis Potosi, Mexico, N21°50.890' W98°56.194' and 43 m above sea level (Fig. 2). Water pH on 3/23/16 was 7.37, conductivity 489 µS, and temperature 26.2 °C.

## Specimens

A poecilid, an epigeomorphic juvenile *Astyanax* and two juvenile (2.7 and 3 cm standard length) troglomorphic *Astyanax* fish were collected from the small cave under the tree on 3/20/16. Four adult (3.48 cm standard length, 0.96 gr; 5.60 cm, 4.82 gr; 6.08 cm, 4.63 gr; 6.68 cm, 6.49 gr in fixed fish) troglomorphic fish were collected from the well on 3/23/16. Juvenile specimens were measured and sacrificed in the field immediately after collection and deposited in 100% ethanol. Adults were taken alive to the laboratory where behavioral experiments were conducted the following day. Specimens were then measured, weighed, euthanized and deposited in formalin 10%. In two specimens the skull cap was opened to examine brain morphology. Collecting permit # SGPA/DGVS/02438/16 from Secretaría del Medio Ambiente y Recursos Naturales, México, was issued to Patricia Ornelas García. Photographs were taken on the field with a Canon EOS100 camera.

## Histology

Optic capsule was extracted with the help of a scalpel and dissection needles under a dissection microscope, embedded in paraffin, and cut into 5-µm sections using a microtome. Staining was done with hematoxilin-eosin as in Espinasa et al. (2001).

## Olfaction

Four adult troglomorphic Chiquitita fish were deposited in a fish tank of 50 × 25 × 10 cm (total volume=12.5 L) with cave water and acclimatized for 24 hrs. Two 50-ml syringes were attached to opposite ends of the tank and connected to medical solution administration tubing containing a Luer stopper to control solution flow (Baxter, Thetford, UK). On one syringe the following protocol was performed: 50 ml of a solution

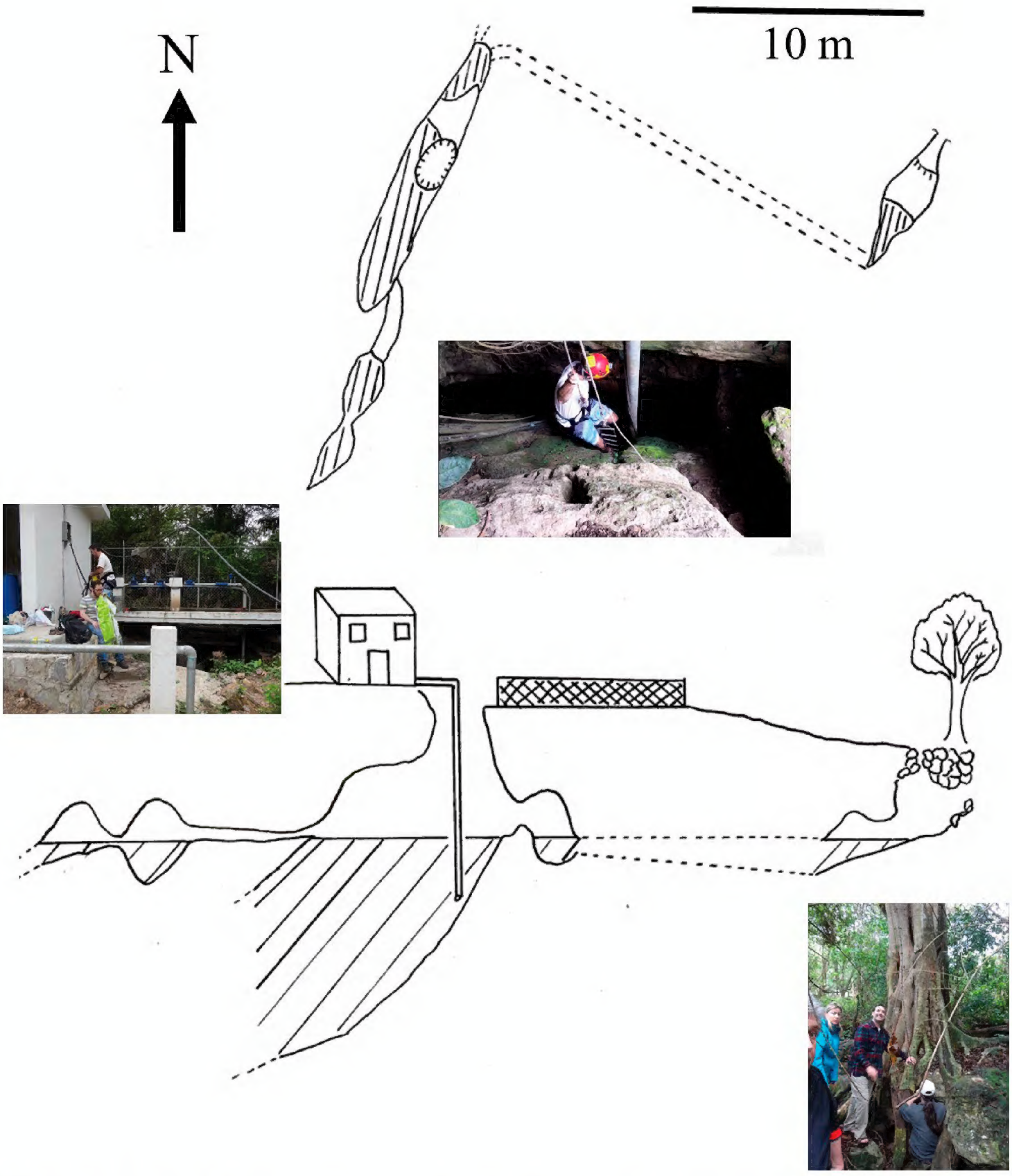


**Figure 1.** Chiquitita Cave map. The accessible and explored cave system is composed of a pit from which locals pump water out and a small chamber under the roots of a tree. Photographs from left to right are: **1** The pump facility with the pipe going into the pit **2** Descending into the pit **3** Entrance to the small chamber under the tree.

with $10^{-12}$ M of Alanine over a 2 min period was allowed to perfuse into the tank. Then 3 min were allowed to pass without adding anything. Then 50 ml of a solution $10^{-11}$ M was added over a 2 min followed by the 3 min of rest. This was repeated for solutions $10^{-10}$ and $10^{-6}$ M. On the other syringe, water was allowed to perfuse at the same rate and times. Solution flow from the experimental and control syringes was initiated si-

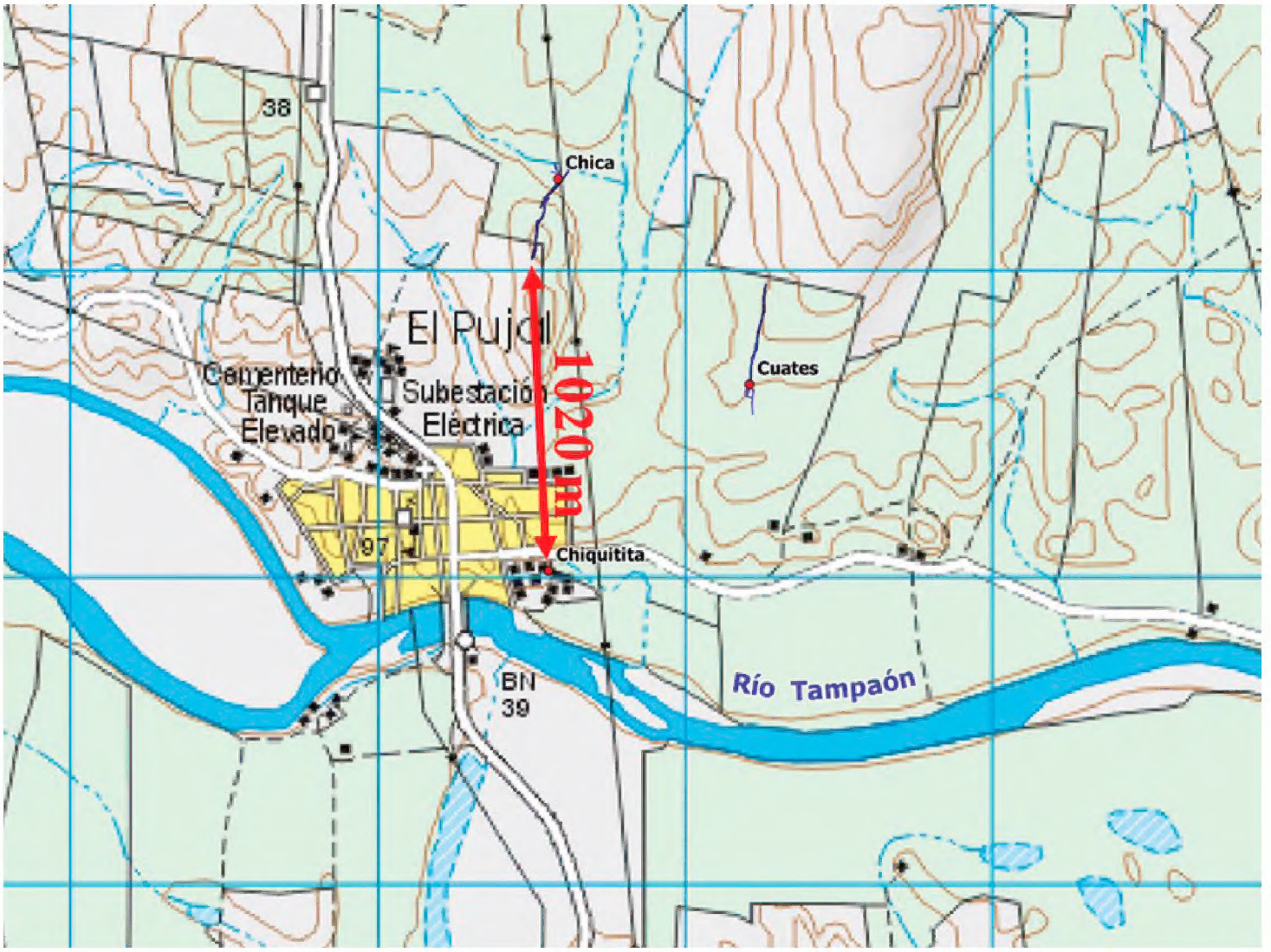


**Figure 2.** Topographic map of El Pujal area, in the southern-most Sierra de El Abra. Overlaid is the line topography of Chica, Cuates and Chiquitita Caves.

multaneously, and the experiment was filmed from the side using a Sony DCR–SR200 Handycam camcorder. Cavefish typically swim parallel to the ground and following one wall of the fish tank until the end of the tank, where they turn either on a 180 degree turn or a 90 degree to follow the next side of the tank. Feeding behaviour is characterized by sharp 360 degree turns around the food source, swimming in circles, or by biting the ground or food source at an angle of about 45°to the ground. For this test, number of sharp, 360 degree turns and bites in the area adjoining the tubing where water (control) or alanine (experimental) was being perfused were counted. Likewise number of turns or bites were counted on the experimental tube area during the rest periods when nothing was perfused. A Mann-Whitney test was perform to compare among conditions.

## Fat deposits

Abdominal dissections were performed in the six Chiquitita Cave individuals with the help of a scalpel, micro-scissors and dissection needles. A flap of skin of the left side of the body, between the base of the opercula and the anal fin was raised so as the expose

the abdominal contents. An image of the abdominal cavity is recorded. From this image the portion of the abdominal lining with fat deposits is measured and compared to the total area of the abdomen so as to provide the percentage of abdominal lining covered with fat deposits. For comparison, 5 individuals from Boquillas river and 4 from Rascon surface fish were analysed. A Mann-Whitney test was then performed.

### DNA sequencing

Previous mitochondrial DNA analyses has shown the presence of two broadly different clades that have been dubbed as lineages A and B or new and old by some authors (Gross 2012). For this study the mitochondrial 16S rRNA was sequenced. Four Chiquitita Cave individuals were analysed. For comparison, DNA was also sequenced from individuals from other locations. Specimens from localities with reported lineage "A" specimens included 2 Comandante surface river, 2 Molino Cave, 2 Caballo Moro Cave, 2 Pachón Cave and 1 Chica cave. Specimens from localities with lineage "B" included 4 Rascon surface stream, 2 Tamasopo surface stream, 2 Sabinos Cave, 4 Tinaja Cave, and 1 Curva cave. Genomic DNA samples were obtained following standard methods for DNA purification using Qiagen's DNeasy® Tissue Kit, by digesting a fin clip of the individual in the lysis buffer. Markers were amplified and sequenced as a single fragment using the 16Sar (CGCCTGTTTATCAAAAACAT) and 16Sb (CTCCGGTTTGAACTCAGATCA) primer pair for 16S rRNA (Edgecombe et al. 2002). Amplification was carried out in a 50 µl volume reaction, with QIAGEN Multiplex PCR Kit. The PCR program consisted of an initial denaturing step at 94 °C for 60 sec, 35 amplification cycles (94 °C for 15 sec, 49 °C for 15 sec, 72 °C for 15 sec), and a final step at 72 °C for 6 min in a GeneAmp® PCR System 9700 (Perkin Elmer). PCR amplified samples were purified with the QIAquick PCR purification kit and directly sequenced by SeqWright Genomic Services. Chromatograms obtained from the automated sequencer were read and contigs made using the sequence editing software SequencherTM 3.0. All external primers were excluded from the analyses. BLAST was used to identify GenBank sequences that resemble the specimens. Sequences were aligned with ClustalW2.

## Results

Chiquitita cave, barely 200 m from Tampaón River, is the southern-most cave in the Sierra de El Abra (Fig. 2). The vertical pit and adjoining small cave are a resurgence during the rainy season as evidenced by the stream bed coming out of these caves, which was dry at the time of exploration. Water level in the cave was estimated to be 37 m above sea level. Tampaón River is reported to be 28.5 m by Mitchell et al. (1977). During the rainy season, the about 10 m vertical difference should be a trivial barrier for aquatic organisms. Evidence of this was the presence of typical surface poecilids and

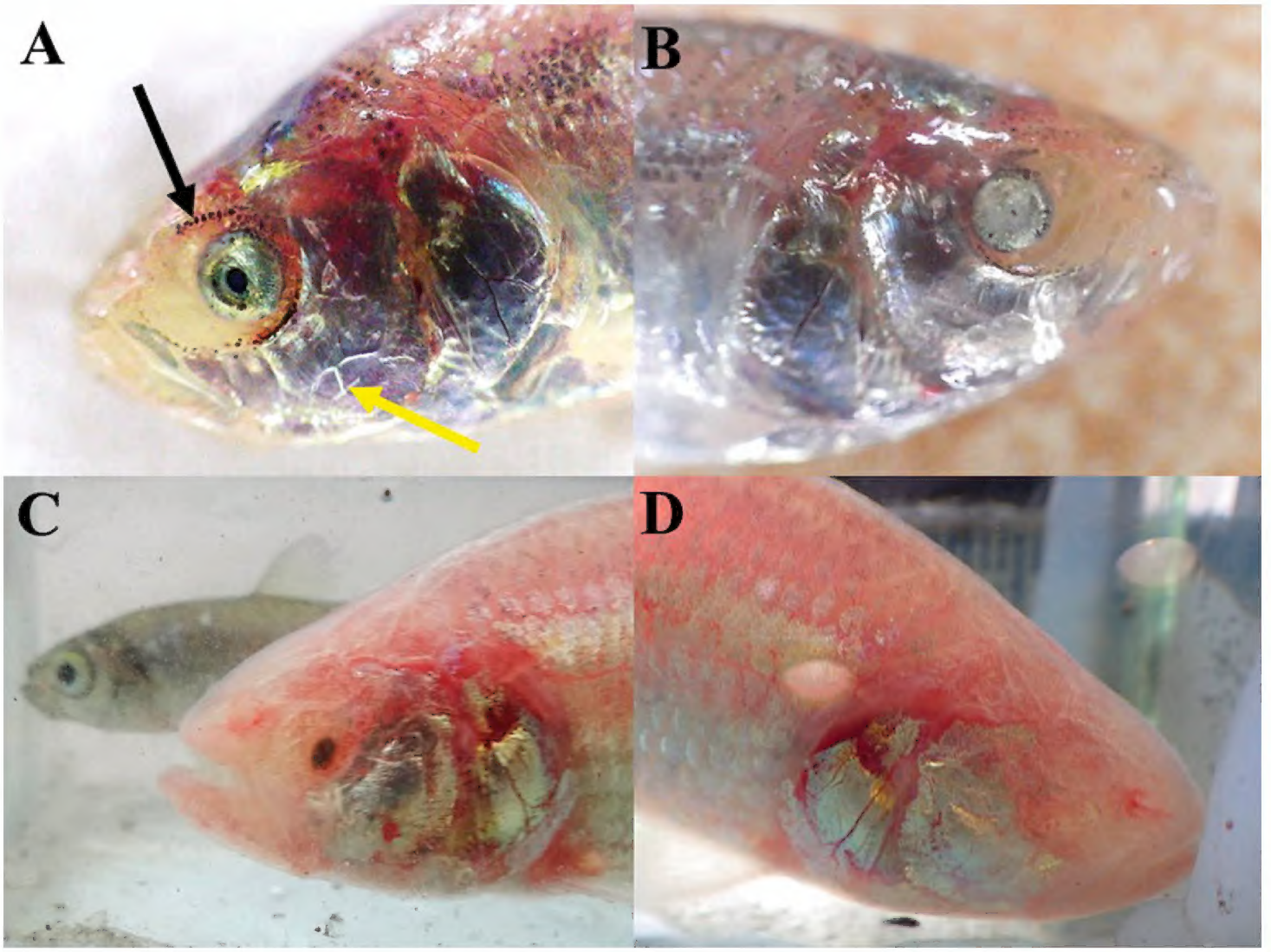


**Figure 3.** High variability in the eye and pigmentation level within the population inhabiting Chiquitita Cave. **A** eye size reduced **B** pupil closed **C** in the foreground a troglomorphic fish with reduced and embedded eyes and in the background a pigmented fish with large eyes **D** eyes and pigment mostly absent. Black arrow highlights pigmented cells in some troglomorphic fish and yellow arrow highlights fragmentation of the suborbital bone III.

a school of surface *Astyanax*. That this cave is part of a very large underground water conduit system is suggested by the fact that water is being continuously pumped out for human use without depleting its water.

Apart from the epigean organisms, Chiquitita Cave is inhabited by mysid troglomorphic shrimps, presumably *Speleomysis quinterensis*, and a population of troglomorphic *Astyanax*. Fish were highly depigmented and were easily differentiated from the surface congeners by their characteristic pinkish-white coloration. There was high variability in the eye, with individuals having phenotypes such as small eye size (Fig. 3A), closed pupil (Fig. 3B), embedded eye (Fig. 3C), and eye mostly absent (Fig. 3D). Introgression between the surface morph and the cave morph is suggested by the presence of individuals that are highly depigmented, but with eyes (Fig. 4B) or individuals with pigment and reduced eyes (Fig. 3A–B).

When the eye capsule was histologically examined in the fish with the smallest eye remnant, it was noted that there was no lens. Retinal layers were disorganized and present only in small sections of the eye capsule (Fig. 5). With such level of eye degeneration, it

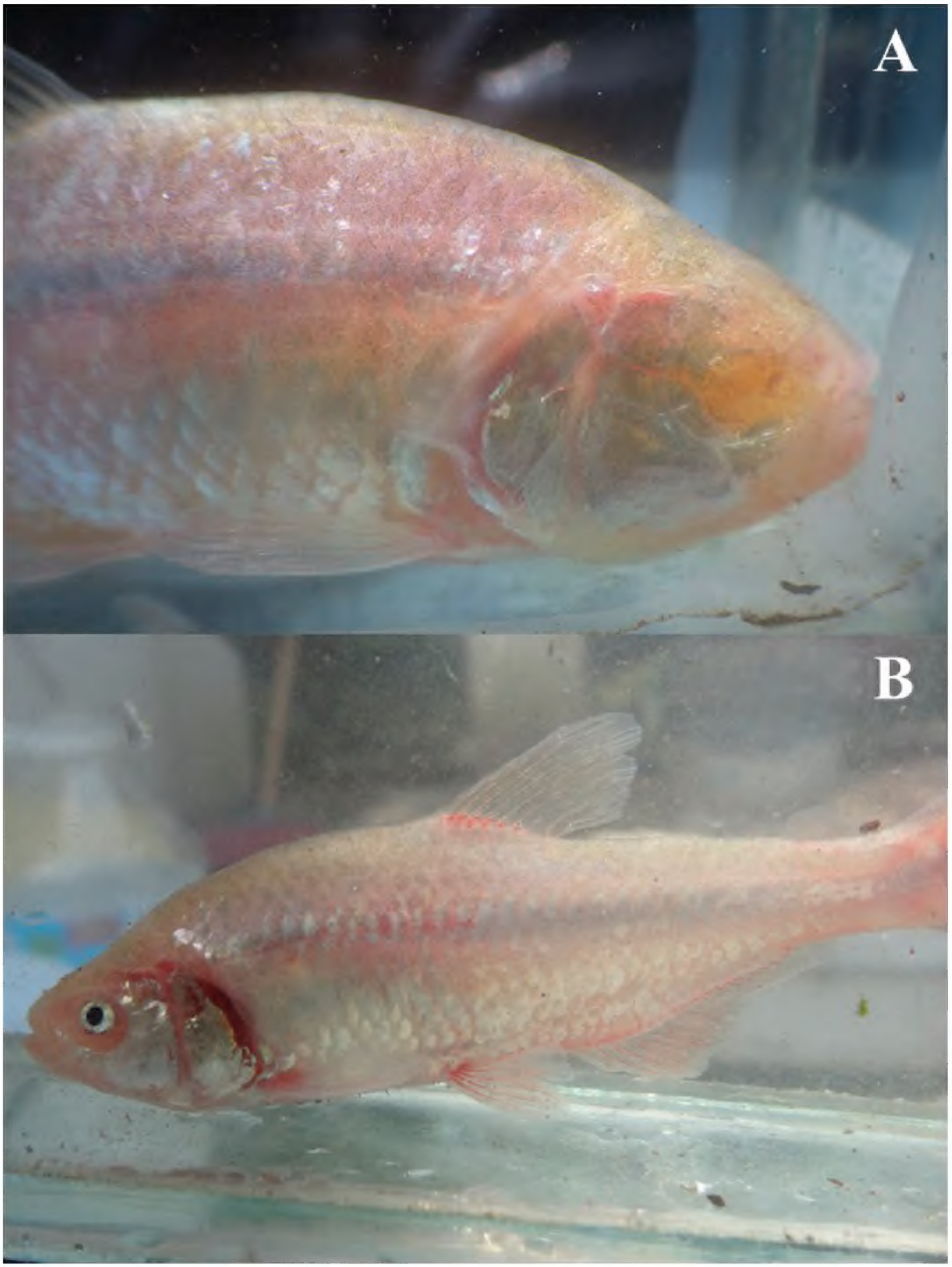


**Figure 4.** Variability in the correlation between eye and pigment may suggest introgression between the surface morph and the cave morph as evidenced by the presence of individuals that are highly depigmented, and without eyes (**A**) or individuals that are also highly depigmented but with eyes (**B**). For the other combinations of eye and pigment see Figure 3.

is likely that these fish are effectively blind. Depigmentation in at least some of the individuals is not due to an albinism mutation because some remnants of the eyes are black (Fig. 5B) and skin has some small level of pigmentation.

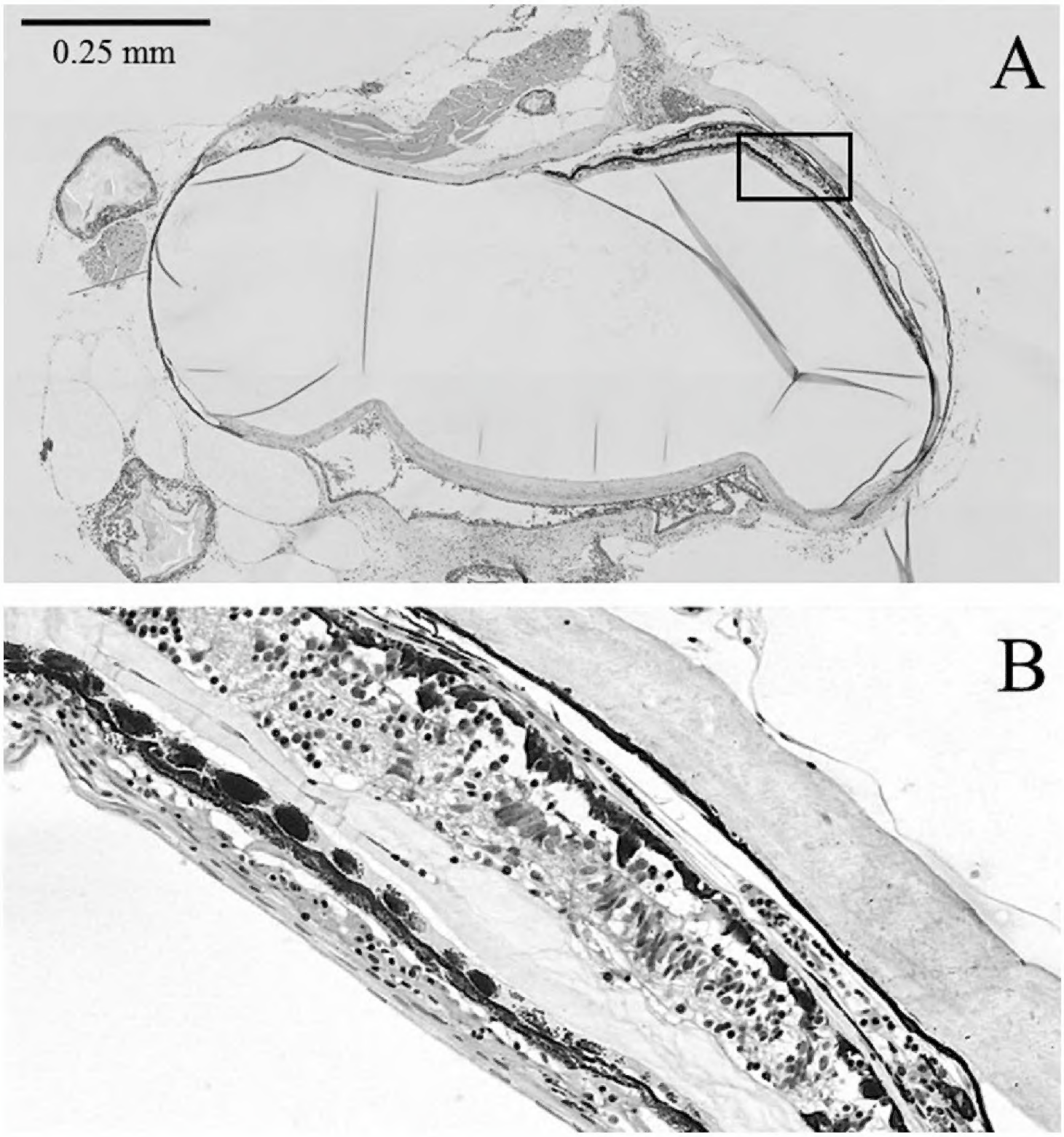


**Figure 5.** Eye histology in one of the fish with most degenerated eyes. **A** eye capsule. Notice the absence of lens. **B** retina. Notice the high disorganization of vestigial layers, which are for the most part unrecognizable when compared to surface fish retinal layers.

The maximum length of the optic lobes was small (1.57 and 1.75 mm) in comparison to their prosencephalon (2.26 and 2.46 mm), thus in the Chiquitita cave specimens, optic lobes were only 70 and 71% the length of the prosencephalon. Based on Espinasa et al. (2001), epigeomorphic *Astyanax* with this length of optic lobes are proportionally larger, being 90% the length of their prosencephalon. Such reduction of the optic lobes is in accordance with reports for troglomorphic *Astyanax* (Wilkens 1988).

These Chiquitita cavefish have other troglomorphic characters previously described for *Astyanax*. For example, they have natural bone fragmentation of the suborbital bones (Gross et al 2016), as highlighted by the yellow arrow in Figure 3A. Likewise

it has been reported that *Astyanax* cavefish have an enhanced ability for fat storage (Hueppop, 1989). In the troglomorphic individuals of Chiquitita Cave, increased subdermal fat reserves was also observed (P=0.021). In the Chiquitita Cave specimens, an average of 32.8% (±19.1 StDev) of the lining of the abdomen is covered by fat deposits, while in surface fish it is 19.9% (±14.4 StDev).

Chiquitita cavefish also appear to have enhanced olfaction capabilities. During perfusion at $10^{-12}$ M of Alanine, 2 turns and 0 bites were recorded on the experimental side. At $10^{-11}$ there were 6 turns and 2 bites. At $10^{-10}$ there were 7 turns and one bite, and at $10^{-6}$ there were 9 turns and 7 bites. During those same periods in the control side, where water was being perfused, there were 1, 0, 2 and 3 turns, with not a single bite. In the experimental side, during the rest period when nothing was being perfused there were 0, 1, 1, and 2 turns, with not a single bite. The total number of turns (15) in the area of the tubing perfusing Alanine concentrations equal or lower than $10^{-10}$ was significantly higher (P=0.0047) than the number of turns (3) in the control tubing perfusing water, or in the side of the experimental tubing (2) during resting time when nothing was being perfused (P=0.0016). This supports that feeding behaviour was being induced by smell at perfused concentrations equal or lower than $10^{-10}$ M of Alanine. Hinaux et al. (2016), using a different set-up from ours (one-month-old juveniles of about 5-6 mm in 9 cm wide × 13 cm long tanks containing 150mL) showed that both surface and cavefish had a strong reaction at perfused concentrations of $10^{-5}$ M of Alanine, but there was a threshold at $10^{-6}$ M when surface fish stopped reacting. In their set-up cavefish still had strong reactions even at concentrations of $10^{-10}$ M. While experimental conditions are different in this study from Hinaux (adult vs juveniles, in 12.5L vs 150mL), data supports that Chiquitita cavefish may be responsive to perfused odorants at low concentrations levels. Remarkably, only sharks have been reported to present such sensitivity to amino acids with, for example, $10^{-11}$ M alanine eliciting electro-olfactogram responses in the hammerhead shark (Tricas et al. 2009).

DNA amplification of the mitochondrial 16S rRNA produced a 572 bp sequence. Results showed that three Chiquitita specimens had identical haplotype to the surface and cave populations belonging to the "A" linage, to which the Chica Cave, Pachón Cave, and local surface *Astyanax* belong (Fig. 6). The fourth Chiquitita cave individual differed by a single base. When compared against the "B" lineage surface fish from Rascon and Tamasopo, Chiquitita specimens differed by 2-3 bp. When compared against the "B" lineage of cave fish from Sabinos, Tinaja and Curva, they differed by 5-6 bp (red arrows on Fig. 6). It is thus supported that Chiquitita specimens have a mitochondrial DNA most closely related to the "A" lineage, with some specimens being identical to individuals from Chica cave.

## Discussion

The first cave to be described with a troglomorphic population of *Astyanax mexicanus* was Chica cave. Since then, cave *Astyanax* has arguably become among the most influential

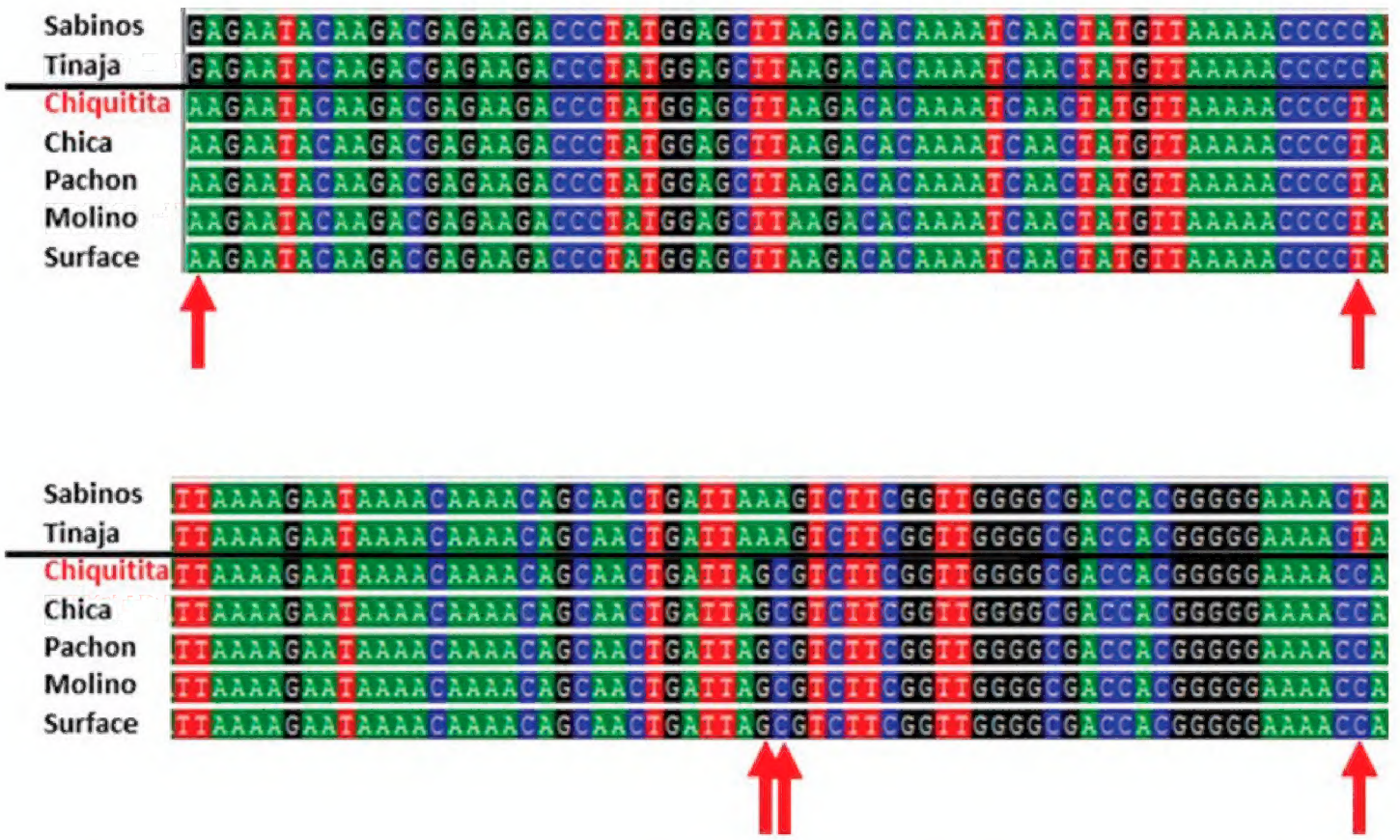


**Figure 6.** Fragment of the mitochondrial 16S rRNA. Individuals from Chiquitita Cave have identical sequence to members of the "**A**" lineage (Chica cave, Pachón cave, Molino cave and Rio Comandante surface river). Members of the "**B**" lineage (Sabinos cave and Tinaja cave) have 5-6 bp disagreements in this fragment, indicated by red arrows.

model system for the study of evolutionary development and genomics in cave biology. A total of 29 caves are now known to be inhabited by these cavefish (Mitchell et al. 1977). Chica population remains one of the most cited among them. The Chica population is special in that it is composed of a mixed population of troglomorphic and epigeomorphic individuals, which may be hybridizing with each other. Somehow counterintuitively, the most epigeomorphic individuals are not found near the entrance, but instead they are in the deeper pools and in particular at the final sump. Since this final sump is almost at the same altitude as the nearby Tampaón surface river, it has been proposed that surface fish are entering at a spring near Tampaón and are then dispersing through the continuous underwater conduits that reach Chica's final sump (Mitchell et al. 1977). The more troglomorphic individuals are in perched pools, partially isolated vertically from the surface fish.

Mitchell et al. (1977) had proposed that the point of entrance of these surface fish was at some tinajas in the town of El Pujal, just 200 m from Tampaón River. When trying to confirm this hypothesis, Chiquitita Cave was discovered just 45 m from these tinajas. Chiquitita cave is only 1020 m at 357°(from true N) to the sump in Cueva Chica (Fig. 2). Chiquitita Cave is a resurgence active during the rainy season and part of an apparently very extensive system of underwater passages of which a minimal part can be explored without the use of cave scuba diving techniques. Surface fish probably have easy access to this resurgence during the rainy season as there are minimal vertical barriers between this cave and the high level of Tampaon River. Evidence for this is the

presence of a large school of surface *Astyanax* inside the cave as well as other surface species swimming in the penumbra.

Chiquitita Cave is also host to a population of troglomorphic fish. These fish showed variability in their eye development and pigmentation level like in the Chica cave, consistent with introgression. Nonetheless, the most troglomorphic fish where essentially devoid of pigment and eyes and presented other troglomorphic features such as fractured suborbital bones, increased subdermal fat reserves, and enhanced olfaction capabilities. In essence, some fish at Chiquitita Cave were essentially as troglomorphic as the most cave adapted individuals in Chica Cave or other El Abra populations.

Population genetics analyses performed on the *Astyanax mexicanus* cave and surface system have demonstrated the co-existence in the El Abra region of two mitochondrial haplotypes, A and B, initially defined after the ND2 gene sequence (Dowling et al. 2002; Strecker et al. 2003, 2004; Ornelas-Garcia et al. 2008). These mitochondrial DNA data have been used to propose evolving scenarii on the relatedness of *Astyanax* cave and surface populations in the region (Fumey et al. 2018). Here, we used 16S rRNA mitochondrial sequence analysis to start documenting the genetic lineage of the newly discovered Chiquitita cavefish population. We found that this mitochondrial DNA marker establishes Chiquitita as belonging to the "A" lineage, together with Molino, Caballo Moro, Pachón, and Chica cavefish, as well as the local surface fish streams. This result is consistent with the possibility that there could be continuous passages and therefore exchanges between the two very close caves of Chica and Chiquitita. Hence, the Chiquitita Cave troglomorphic population may essentially be seen as an extension of the Chica population. The distance of about 972 m between the two caves, at essentially the same altitude, should not pose an effective barrier to prevent individuals from dispersing between them. Likewise surface fish entering during the rainy season at the spring of Chiquitita Cave may be able to disperse easily towards the final sump of Chica Cave (see Fig. 2).

An important ecological difference between the two caves is that while Chica Cave has a large bat colony that provides enormous amounts of bat guano and carcasses as a food source, Chiquitita Cave must be more challenging. There seems to be minimal input of food sources in comparison, especially beyond the twilight where no more surface insects may fly in. This may be specially challenging for non-cave adapted surface fish.

Possible impacts and threats to this new population are mainly human-derived. This cave has a pipe from which water is extracted for local consumption. Nonetheless the cave appears to be connected to a large aquifer and despite constant usage, the water table appears to recharge readily. Pachon Cave, the most studied *Astyanax* cave population, also has a pipe for water extraction and the population has remained large. Being connected to a large aquifer probably translates into this pipe only potentially having a very local effect to the proportionally few individuals in the area. Of more concern is that the cave is located under the town of El Pujal and any chemical or toxic waste may find its way into the local aquifer.

## Conclusions

Despite the latest surge of interest in the *Astyanax* model, not a single new cave locality had been described for *Astyanax* cavefish in El Abra region over the last 40 years. We describe here a new cave locality, Chiquitita Cave, inhabited by troglomorphic *A. mexicanus*. Chiquitita Cave is a resurgence found at the southern-most edge of the Sierra de El Abra, very close to Tampaón River. While some of its individuals are apparently fully troglomorphic in nature, it is a mixed population with surface individuals probably hybridizing with the troglomorphic population. It is also likely that there are continuous underwater passages between Chica and Chiquitita cave, thus potentially allowing for both troglomorphic and surface fish to migrate between both localities.

## Acknowledgments

We would like to thank Patricia Ornelas-Garcia who obtained a collective collection permit. Maria Elina Bichuette and an anonymous reviewer for their comments on the manuscript. Thanks to all group members who participated to the March 2016 field trip: D. Casane, L. Devos, C. Hyacinthe, S. Père, K. Pottin, E. Queinnec, V. Simon. This study was supported by Marist College and its School of Science (to LE), an ANR grant [BLINDTEST] and a FRM grant [Equipe FRM] (to SR), and a collaborative exchange program [Ecos-Nord] to SR and Patricia Ornelas-Garcia.

## References

Avise JC, Selander RK (1972) Evolutionary genetics of cave-dwelling fishes of the genus Astyanax. Evolution 26(1): 1–9. https://doi.org/10.1111/j.1558-5646.1972.tb00170.x

Edgecombe GD, Giribet G (2002) Myriapod phylogeny and the relationships of Chilopoda. In: Llorente Bousquets, JE and Morrone JJ (Eds.), Biodiversidad, Taxonomía y Biogeografía de Artrópodos de México: Hacia una síntesis de su conocimiento Prensas de Ciencias, Universidad Nacional Autónoma de México, Mexico D.F., pp. 143–168.

Elliott WR (2015) Cave ecology and biodiversity in the Sierra de El Abra Region. In: Keene AC, Yoshizawa M, McGaugh SE (Eds) Biology and Evolution of the Mexican Cavefish. Academic Press (Elsevier), Amsterdam, Chapter 3, 59–76. https://doi.org/10.1016/B978-0-12-802148-4.00003-7

Elipot Y, Legendre L, Père S, Sohm F, Rétaux S (2014) *Astyanax* transgenesis and husbandry: how cavefish enters the laboratory. Zebrafish 11: 291–299. https://doi.org/10.1089/zeb.2014.1005

Espinasa L, Rivas-Manzano P, Espinosa Pérez H (2001) A new blind cave fish population of the genus *Astyanax*: geography, morphology and behavior. Environmental Biology of Fishes 62: 339–344. https://doi.org/10.1007/978-94-015-9795-1_27

Fumey J, Hinaux H, Noirot C, Thermes C, Rétaux S, Casane D (2018) Evidence for Late Pleistocene origin of *Astyanax mexicanus* cavefish. BMC evolutionary biology 18(1): 43. https://doi.org/10.1186/s12862-018-1156-7

Gross JB (2012) The complex origin of Astyanax cavefish. BMC Evolutionary Biology 12: 105–10. https://doi.org/10.1186/1471-2148-12-105

Gross JB, Stahl BA, Powers AK, Carlson BM (2016) Natural bone fragmentation in the blind cave-dwelling fish, *Astyanax mexicanus*: candidate gene identification through integrative comparative genomics. Evolution & development 18(1): 7–18. https://doi.org/10.1111/ede.12131

Hinaux H, Devos L, Blin M, Elipot Y, Bibliowicz J, Alié A, Rétaux S (2016) Sensory evolution in blind cavefish is driven by early embryonic events during gastrulation and neurulation. Development 143(23): 4521–4532. https://doi.org/10.1242/dev.141291

Hueppop K (1989) Genetic analysis of oxygen consumption rate in cave and surface fish of Astyanax fasciatus (Characidae, Pisces). Further support for the neutral mutation theory. Mémoires de Biospéologie XVI: 163–168.

Hubbs CL, Innes WT (1936) The first known blind fish of the family Characidae: A new genus from Mexico. Occasional Papers of the Museum of Zoology of Michigan 342: 1–7.

Jeffery WR (2001) Cavefish as a model system in evolutionary developmental biology. Developmental biology 231(1): 1–2. https://doi.org/10.1006/dbio.2000.0121

Jeffery WR (2012) *Astyanax mexicanus*: A model organism for evolution and adaptation. In White WB and Culver DC (eds). Encyclopedia of Caves Second edition. Academic Press, Waltham, MA: 36–43. https://doi.org/10.1016/B978-0-12-383832-2.00006-2

Kopp J, Coppola J, Espinasa L (2017) Phylogeographical convergence between two troglobitic organisms in the Sierra de El Abra. Memoirs of the 5th Astyanax International Meeting. Santiago de Queretaro, Mexico.

Mitchell RW, Russell WH, Elliott WR (1977) Mexican eyeless characin fishes, genus *Astyanax*: environment, distribution, and evolution. Special Publications Museum Texas Tech University 12: 1–89.

Strecker U, Bernatchez L, Wilkens H (2003) Genetic divergence between cave and surface populations of Astyanax in Mexico (Characidae, Teleostei). Molecular Ecology 12: 699–710. https://doi.org/10.1046/j.1365-294X.2003.01753.x

Tricas TC, Kajiura SM, Summers AP (2009) Response of the hammerhead shark olfactory epithelium to amino acid stimuli. Journal of Comparative Physiology A. Neuroethology, Sensory, Neural, and Behavioral Physiology 195: 947–954. https://doi.org/10.1007/s00359-009-0470-3